composition to a carrier and then contacting with the skin. The dose of antigen protein in a vaccine composition generally ranges from about 0.1 ng to about 100 mg per administration, preferably from about 1 μg to about 1 mg per administration. On the other hand, the amount of adjuvant to be formulated is determined depending on the type and dose of immunological antigen to be used. The carrier for the vaccine composition can be an absorptive carrier, such as lint, and a conventional carrier for applying the dose as a paste or spray.

Oral, percutaneous or intranasal inoculation is highly preferred for the vaccines exemplified below, both in terms of effectiveness of vaccine and convenience of inoculation procedure. Examples of such vaccines include, but are not limited to, influenza vaccine, pertussis vaccine, diphtheria vaccine, rotavirus vaccine, measles vaccine, rubella vaccine, mumps vaccine, *Helicobacter pylori* vaccine, enterohaemorrhagic *Escherichia coli* (EHEC) vaccine, *Chlamydia* vaccine, *mycoplasma* vaccine, coccidium vaccine, AIDS vaccine, malaria vaccine, and schistosome vaccine, etc.

These vaccines can be administered alone or in combination with other agents, by mixing more than two of them and inoculating them simultaneously, for example, like pertussis-diphtheria-tetanus trivalent vaccine or measles-rubella divalent vaccine. One of the reasons why oral and intranasal inoculations are preferred is that mucous membranes of the respiratory tract and digestive tract are important sites of early stage infection. Such inoculations can prevent infection at a stage as early as possible by activating the immunogenic system in the local mucous membrane with the inoculation of a vaccine comprising an appropriate adjuvant possessing potent immuno-stimulating activity. Further, for some vaccinations, such as vaccinations against Malaria *Plasmodium*, which are typically performed in regions without sufficient medical facilities, it is advantageous to select vaccination routes such as oral, percutaneous and intranasal inoculation routes because such vaccines can be administered without the help of medical staff such as physician or nurse. In addition, percutaneous inoculation is desirable, because the vaccination can be halted as may be demanded when adverse side effect or the like encounters.

Brief Description of the Drawings

Figure 1 is a graph showing the binding capacity of attenuated cholera toxin to ganglioside GM1.

This figure shows an example of a confirmation test for attenuation, which was conducted during the process of producing an adjuvant of the invention by attenuating cholera toxin with formalin treatment. In this figure, the ordinate axis indicates the binding capacity to ganglioside GM1, which is the receptor (O.D. at 410 nm in ELISA), and the abscissa axis indicates toxin concentration (ng/ml).

Figure 2 is a graph showing the effect of attenuated cholera toxin to enhance production of anti-influenza virus antibody in the nasal mucous membrane by the secondary response.

This figure shows the antibody titer in nasal wash, resulting from the intranasal administration of a vaccine preparations of the present invention comprising an influenza vaccine antigen. The ordinate axis indicates attenuated cholera toxins treated under a variety of conditions and the abscissa axis indicates the concentration (μg/ml) of anti-HA IgA antibody in the nasal washes.

Figure 3 is a graph showing the effect of attenuated cholera toxin to enhance the production of anti-influenza virus antibody in the serum by the secondary response.

This figure shows the antibody titer in the serum, resulting from the intranasal administration of a vaccine preparation of the present invention comprising an influenza vaccine antigen. The ordinate axis indicates attenuated cholera toxins treated under a variety of conditions and the abscissa axis indicates the concentration (μg/ml) of anti-HA IgA antibody in the serum.

Figure 4 is a graph showing the immuno-stimulating activity of attenuated toxin.

This figure shows the antibody titer in the nasal wash or in the serum in the secondary response, resulting from the intranasal administration of an influenza vaccine of the present invention, containing cholera toxins of which residual toxin activities are different to each other as the adjuvants. The ordinate axis indicates a relative value (■) of anti-HA IgA antibody titer in the nasal wash

of the experiment group, where attenuated cholera toxin was used, to anti-HA IgA antibody titer in control group, where natural cholera toxin was used, or a relative value (○) of anti-HA IgG antibody titer in the serum of the experiment group, where attenuated cholera toxin was used, to anti-HA IgG antibody titer in control group, where natural cholera toxin was used. The abscissa axis indicates a relative value of residual toxic activity of attenuated cholera toxin used in the test to the activity of natural toxin (measured by Y-1 cell morphologic transformation test).

Figure 5 is a graph showing the synergistic effect of dual adjuvants.

This figure shows immunological responses resulting from the intranasal administration of an influenza vaccine that contains the attenuated cholera toxin of the invention and a conventional *E. coli* heat-labile toxin B subunit as the adjuvants. The ordinate axis indicates the type and amount of adjuvant used in the test and the abscissa axis indicates the degree of immunological response measured by mouse foot swelling test.

Best Mode for Carrying out the Invention

Examples of the present invention are illustrated below, but the present invention is not to be construed as being limited thereto.

Example 1 – Preparation of cholera toxin:

The cholera toxin was produced according to the method described by Finkelstein et al. (R. A. Finkelstein et al. J. Infect. Dis., 121, Suppl., S63, 1970).

A cholera toxin-producing bacterium (*Vibrio chorela*; Inaba type 569B strain) was cultured in a semi-synthetic casamino acid medium (liquid medium containing glucose) designed by Finkelstein et al., at 30°C for 20 hours. After the culture was completed, the culture supernatant was subjected to ultrafiltration, using a filter having pores that the cholera toxin molecule (molecular weight; 86,000 Da) could freely go through. A small amount of aluminum hydroxide gel was added to the filtrate. The gel was allowed to adsorb the toxin and then was collected by centrifugation. The toxin was eluted from